shows distinct state 2, 3 and 4 respiration, which can be mimicked in E9.5 WT hearts by incubation with 200 nM CsA. By E11.5 mitochondrial oxygen consumption in WT hearts is not significantly different when compared to E11.5 CypD KO mice or E13.5 hearts.
In addition, supercomplexes were not detectable in WT hearts of E9.5 and E11.5 embryos by native electrophoresis or immunocapturing. This may explain why NADH-cytochrome c reductase activity is not detectable in WT embryos at E9.5 but in E11.5 or E13.5 WT mice. Furthermore, Cx-1 is in its de-active form at E9.5 and becomes activated around E11.5.
Our data suggests that at E11.5 the PTP is closed and Cx-1, Cx- 2 and Cx-3 of the ETC are present, but are not yet arranged in supercomplexes and a coordinated electron transfer is still developing.

939-Pos Board B694
Understanding the Contribution of mtHsp70 towards Mitochondrial Dysfunction in Parkinson's Disease: A Yeast Model
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Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders, characterized by loss of dopaminergic neuron function; leading to bradykinesia and motor dysfunction. It is observed that various genes which have been implicated in the familial and sporadic forms of the disease are involved in pathways which focalize at the mitochondria. This organelle is a central regulator of cell death, and mitochondrial dysfunction is a common phenomenon associated with multiple neurodegenerative diseases. Not surprisingly, a clinical screen identified PD-associated mutations in mitochondrial Hsp70 (mtHsp70), which is a critical protein involved in mitochondria biogenesis and maintenance of homeostasis. In order to specifically understand the role of the mtHsp70 protein, we have developed a *Saccharomyces cerevisiae* model for studying the disease variants in isolation from other players of this multifactorial disease. We generated the analogous mutants R103W and P486S in yeast mtHsp70 which remarkably recapitulated the symptoms of mitochondrial dysfunction in affected neurons including cell death, increased generation of ROS, mtDNA loss and respiratory incompetence. Spectral analysis of a fluorescent carbocyanine dye uptake in isolated mitochondria indicated compromised membrane potential gradients as a cause for dysfunction. At the molecular level, circular dichroism based-thermal melt analyses confirmed the observed *in vivo* aggregation propensity of R103W, while P486S exhibited futile enhanced interaction with J-protein co-chaperone partners thereby resulting in loss of chaperoning activity by mtHsp70. Remarkably, these altered biochemical properties reflect identical defects found by us in the human mtHsp70 variants, thereby confirming a direct involvement of mtHsp70 in PD progression. Thus, by utilizing the significant structural and functional homology between the yeast and human mtHsp70 proteins, and an integrated *in vivo* and *in vitro* approach, we demonstrate the basic underlying mechanisms of mtHsp70 association with PD.

940-Pos Board B695
Amphipathic Tail-Anchoring Peptide is a Promising Therapeutic Agent for Cancer Treatment
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The amphipathic tail-anchoring peptide (ATAP) derived from the human anti-apoptotic protein Bfl-1 is a potent inducer of apoptosis by targeting mitochondria permeability transition. By linking ATAP to an internalizing RGD peptide (iRGD), selective targeting for ATAP to tumor cells could be achieved. Targeting of ATAP-iRGD peptide to tumorigenic cells resulted in mitochondrion-dependent cell death through release of cytochrome c. Confocal fluorescence microscopy showed that Dylight488-labeled ATAP-iRGD could effectively penetrate into cells and distribute along the mitochondria network. Flow cytometry results showed that expression level of integrinαV or integrinαVβ3 receptors is higher in ATAP-iRGD sensitive (DU145, KYSE-150 and PC3) cells, when compared with ATAP-iRGD insensitive (K562 human leukemia cell) cell line. These results indicate that integrins act as a receptor to mediate uptake of ATAP-iRGD into the cancer cells. Studies with xenograft model showed that intravenous injection of ATAP-iRGD could suppress the growth of several human carcinoma cells implemented into the nude mice. Toxicological studies revealed that repetitive intravenous delivery of ATAP-iRGD did not produce significant toxicity in different organs of the SV129 mice. Our data suggest that ATAP-iRGD is a promising agent with high efficacy and limited toxicity for cancer therapy.

941-Pos Board B696
Blebbistatin Delays Mitochondrial Depolarization and Asystole during Myocardial Ischemia, and Prevents Cell Death Upon Reperfusion
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Myosin II inhibitor Blebbistatin is frequently used for heart immobilization during imaging experiments involving ischemia/reperfusion (I/R). Here we investigated the extent to which Blebbistatin modulates critical events during I/R, such as mitochondrial membrane potential depolarization ($\Delta\Psi_m$), asystole, and infarction. Langendorff-perfused rabbit hearts were subjected to 60-90 minutes of ischemia and 180 minutes of reperfusion in the absence (control) or in the presence of Blebbistatin (5.7 μM). We monitored $\Delta\Psi_m$ depolarization using spectral analysis of confocal images yielded by cationic fluorescent dye TMRM. We detected the onset of sarcolemmal permeabilization (SP) in individual myocytes as a massive uptake of anionic dye DiBac4(3) or cell-impermeable nucleic acid dye YO-PRO1. Control hearts were briefly perfused with 20 mM potassium at 35 minutes prior to ischemia, and at 30 and 120 minutes of reperfusion to achieve immobilization necessary for confocal imaging. In control hearts, cessation of contraction permitted confocal imaging after 10-15 minutes of ischemia. Global and local bipolar electrograms monitored electrical activity. During ischemia, Blebbistatin significantly ($p<0.01$) and proportionally delayed both $\Delta\Psi_m$ depolarization (50.5 minutes versus 24.0 minutes in control) and asystole (40.2 minutes versus 22.3 minutes in control). Upon reperfusion, a heterogeneous $\Delta\Psi_m$ recovery was observed in both groups, but Blebbistatin largely prevented SP events observed on a massive scale in control hearts. Additionally, Blebbistatin almost completely prevented infarction in this model ($1.9 \pm 3.2\%$ versus $75.0 \pm 7.8\%$ in control by tetrazolium chloride staining). Another electromechanical uncoupler, 2,3-Butanedione monoxime (20 mM), did not prevent SP and infarction despite efficient immobilization. Conclusions: (1) great caution has to be exercised with regard to interpretation of I/R events observed in the presence of Blebbistatin; (2) the mechanism(s) of prominent cardioprotection by Blebbistatin may not be fully explained by immobilization and deserve further study.

942-Pos Board B697
Role of Mitochondria-Cytoskeleton Interactions in Respiration Regulation in Post-Infarct Heart Failure
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The aim of this work is to study structural and functional organization of energy metabolism in an ex vivo model of myocardial ischemia-reperfusion, focusing on the role of mitochondria interactions with cytoskeletal proteins in the regulation of mitochondrial respiration and dynamics. Hearts studied were subjected to perfusion under normoxic, ischemia and ischemia-reperfusion conditions using Langendorff system.
Hemodynamic measurements showed that reperfusion-dependent left ventricular diastolic dysfunction is related to increased affinity of respiration for ADP (low app. Km for ADP). This loss of ADP microcompartmentalization was associated with decreased functional coupling between oxidative phosphorylation and mitochondrial creatine kinase. Previous studies have shown that in healthy adult cardiomyocytes, characterized by high apparent Km for ADP, cytoskeletal protein β2 tubulin co-localises with mitochondria. Ischemia-reperfusion injury induces the displacement of β2 tubulin from mitochondrial position in cardiac muscle fibers with low apparent Km for ADP.
We conclude that ischemia-reperfusion effects on mitochondrial respiration and dynamics could be related to the disruption of mitochondrial outer membrane porin, VDAC, interaction with cytoskeletal proteins such as β2 tubulin.

943-Pos Board B698
Critical Events in Myocardial Ischemia/Reperfusion: Mitochondrial Depolarization Versus Sarcolemmal Permeability
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The critical events determining cell death in the aftermath of myocardial ischemia/reperfusion (I/R) remain poorly understood. Here we investigated